IN THE SPECIFICATION:

Please amend the following paragraphs in the specification:

[097] Neuroblastoma cells (Neuro2A, mouse) were grown in Dulbecco's Eagle medium (Life Technologies - Gibco, UK) ~~supplement~~ supplemented with 10% (V/V) heat-treated foetal calf serum, 2mm glutamine (Life Technologies - Gibco, UK) and 100 units streptomycin-penicillin (Life Technologies - Gibco, UK). The ~~culture~~ cultures were incubated at ~~370C~~ 37°C in a humidified atmosphere containing 8t ~~C02~~ CO_2 and transiently transected using either the $CaPO_4$ **[[,]]** technique or the FuGENE 6™ transfection reagent (~~Roche~~ ROCHE).

[098] Cells were harvested 48h after transfection in 250µl of 10mM β--mercaptoethanol, 4mM EDTA, 5μM coelenterazine in PBS at 4°C during 2 to 4 hours. Cells were rinsed in 1mM EDTA in PBS and harvested in 400μl of hypo-osmotic buffer (20mM Tris-HCl pH 7.5/ 5mM EDTA/ 5mM β-mercaptoethanol with a protease inhibitor cocktail according to the manufacturer, ~~Roche~~ ROCHE), for ~~30 min. to 1h.~~ 30 min to 1 h at 4°C. The cell ~~membrane~~ membranes were broken by passing through a 30 gauge needle and the cellular extract was obtained after microcentrifugation at 13000 rpm for 1h at ~~40C~~ 40°C. The supernatant was harvested for all constructions but ~~SGSA~~ SG5A for which the membrane pellet was further resuspended. Calcium sensitivity chemiluminescent activity was measured in a luminometer (~~Lumat~~ LUMAT LB95501 E&EG ~~Berthold~~ BERTHOLD). Aliquots (10µl) were placed in sample tube (with 90µl of 10mM Tris-HCl pH 7.5) in the luminometer and the light intensity expressed in relative

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light unit (R.L.U.) was measured after the injection of 100µl of 50mM $CaCl_2$/10mM Tris-HC1 pH 7.5 solution.

[099] For CRET measurements, aliquots of extracts from transfected cells were placed in a reservoir chamber and brought into contact with an optic fibre bundle attached to a photon counting camera (~~Photek~~ PHOTEK three-microchannel plate intensified CCD camera: ~~Photek~~ PHOTEK 216). Before capture of signals, light passes through a monochromator allowing the spectral analysis of emitted photons. The acquisition begins 20 seconds before injection of $CaCl_2$ and carries on during 40 seconds after injection of the $CaCl_2$ solution (50mM). For green/blue photons ratio determinations, the same procedure was followed but in this case the system measures the light emitted through blue (450nm) and green (500nm) filters after a beam splitter.

[0102] The turnover times of the different cytosolic proteins were estimated on transient expression in COS7 cells by treatment with puromycin (50µg/ml) for 6h. Ca^{2+}-induced chemiluminescence activities were performed on cellular extract obtained after the reconstitution of aequorin in presence of 5µM coelenterazine. Calcium sensitivity chemiluminescence activity was measured in a luminometer (~~Lumat~~ LUMAT LB95501 E&EG ~~Berthold~~ BERTHOLD). Aliquots (10µl) were placed in a sample tube (with 90µl of 10mM Tris-HCl, pH 7.5) in the luminometer and the light intensity expressed, in relative light units (RLUs), was measured after the injection of 100µl of 50mM $CaCl_2$/10mM Tris-Hcl pH 7.5 solution. Relative chemiluminescence activities are expressed as a percentage of the activity at the time zero (100%). The results are

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shown in Fig. 5. As seen in Fig. 5, over this period, most fusion proteins presented 30% decrease of activity compared with the 80% loss of apoaequorin when alone.

[0103] Ca^{2+}-induced chemiluminescence activities were performed on cellular extract obtained after the reconstitution of aequorin in presence of 5µM coelenterazine. Calcium sensitivity chemiluminescence activity was measured in a luminometer (~~Lumat~~ LUMAT LP95501 E&EG ~~Berthold~~ BERTHOLD). Aliquots (10µl) were placed in a sample tube (with 90µl of 10mM Tric-HCl, pH 7.5) in the luminometer and the light intensity expressed, in relative light units (RLUs), was measured after the injection of 100µl of different Ca/EGTA solutions. The results are shown in Fig. 6. As seen in Fig. 6, G5A gives a significant signal over background with Ca^{2+} concentrations as low as 38 nM, whereas aequorin needs 28 times more calcium (1M) to yield a comparable signal.

For Chimeric GFP-aequorin as bioluminescent Ca^{2+} reporters at the single cell level

Concerning the invention of chimeric GFP-aequorin calcium sensitive bioluminescent reporters, we have developed new applications and we have some preliminary ~~datas~~ data about sensitivity of GFP-aequorin proteins to Ca^{2+} ions.

EXAMPLE 8

Ca^{2+} sensitivity of G5A and SG5A: Calibration curves between bioluminescent signals and Ca^{2+} concentrations

Measurements of Ca^{2+} sensitivity of two constructs G5A and SG5A were performed on cellular extracts obtained after the reconstitution of aequorin in presence of 5µM colenterazine. Calcium chemiluminescence activity was measured in a luminometer (~~Lumat~~ LUMAT LB95501 E&EG ~~Berthold~~ BERTHOLD). Aliquots (10µl)

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were placed in a sample tube with 90 µl of 10mM ~~Tris.HCl~~ Tris-HCl pH 7.5 in the luminometer and the light intensity expressed, in relative light units (RLUs), was measured after the injection of 100ml of different Ca/EGTA solutions (Molecular Probes Calcium Calibration Buffer Kit). Figure 7 shows the Ca^{2+} response curve of G5A, SG5A and aequorin. The curves represent the relationship between the ratio L/Lmax and [Ca2+]. L is the rate of RLUs at any given [Ca2+] and Lmax is the rate of RLUs at saturating [Ca2+]. These results show a much higher affinity for Ca^{2+} of the various forms of GFP-aequorin than aequorin.

EXAMPLE 9

New applications of GFP-aequorin reporters

Adenoviral vectors with GFP-aequorin were developed. Using these new constructs, dissociated neurons from rat spinal cord in culture can be transfected with higher efficiency. Figures 8 and 9 depict Ca^{2+}-induced bioluminescent signals detected at the single cell level in dissociated neuronal cells. Neuronal cells infected by adenoviral vectors with G5A (Fig. 8) or SG5A (Fig. 9) were pre-incubated with 5µM coelenterazine in a Ca^{2+}-free buffer. Intensities of fluorescence and bioluminescence activity are translated in pseudocolors. Representative pictures of the chosen fields are shown after the addition of 5mM and 2.5mM of $CacCl_2$, respectively, for Figures 8a-c & 9a at 12 and 9 seconds. Figures 8d-e and 9b were obtained after addition of ionomycin and high concentration of $CaCl_2$ (100mM).

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EXAMPLE 10

Expression of GFP-aequorin reporters *in vivo* in *Xenopus* embryos and measurement of calcium activities

Calcium signaling during early and late embryogenesis in *Xenopus* was studied. Figure 10 shows representative pattern of luminescence activity illustrating the changes in intracellular calcium during the neural induction after the injection of the GA plasmid at the one cell stage in *Xenopus* embryo. Figure 11 shows a transgenic *Xenopus* larva with GFP-aequorin. These techniques can also be employed with zebrafish and mouse transgenics. These results show that these calcium reporters can be used in a grate variety of organisms or tissues to visualize calcium activity and to measure calcium concentrations.

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